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# Synergistic effects of optical brighteners on the insecticidal activities of Iranian nucleopolyhedrovirus isolates against *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae

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**Abstract**: In order to improve the biological activities ($LC_{50}$ and $LT_{50}$ values) of two geographic isolates of *Helicoverpa armigera* nucleopolyhedrovirus (EAZ-I and EAZ-II), collected from East-Azarbaijan, Iran, some optical brighteners have been combined with the isolates under laboratory conditions to test their activity against early 2nd instar larvae of *H. armigera*. The results demonstrated that the EAZ-I isolate had better insecticidal activity than EAZ-II due to its lower $LC_{50}$ and $LT_{50}$ values of $1.98 \times 10^3$ OB/mL and 122.7 h, respectively. All the optical brighteners used in this study could efficiently enhance virus biological activities, especially Tinopal F-3543 in combination with EAZ-I showing the lowest $LC_{50}$ at 0.2% level ($5.16 \times 10^2$ OB/mL), with 3.84 times higher activity than that of the virus alone. The relative speed of kill demonstrates that all the optical brighteners improved $LT_{50}$ of the isolates, of which Tinopal F-3543 was identified as the most effective one. These findings suggest that the optical brighteners which target PM permeability can be considered as important alternatives to combine with HearNPV formulations in IPM programs.

**Key words**: *Helicoverpa armigera*; nucleopolyhedrovirus; geographic isolates; optical brighteners; insecticidal activity; synergistic effects; $LT_{50}$; $LC_{50}$.

## 1 INTRODUCTION

To overcome increasing problems associated with the strategy of exclusive and indiscriminate use of pesticides, integration of other control techniques have been expanded during the past decades. Exploitation of entomopathogenic viruses is one of the most important integrated control techniques (Dhaliwal and Arora, 1998). Since the nucleopolyhedrovirus HearNPV frequently shows high potential in insect pest control (Mehrvar *et al.*, 2008a, 2008b), it has become an alternative competent in IPM programs, especially to control cotton boll worm, *Helicoverpa armigera* (Rabindra, 2001; Battu *et al.*, 2002; Mehrvar *et al.*, 2009). However, to date, the use of NPVs as microbial control agents has been limited by a number of factors, particularly the non-availability of appropriate methods to scale up the virus production process to the desired levels, shorter persistence, and absence of stable and effective formulations which consequently limits their commercial exploitations. Besides, low speed of kill is another important element which considerably limits their usage. So, any chemicals which can effectively enhance the virus lethal time in the insect body should be taken into considerations. Optical brighteners are one of these chemicals (Jayachandran *et al.*, 2000) which affect the peritrophic membrane (PM) of the insect midgut and disrupt its structure and function which, in turn, cause synergistic effects on biological activities of NPVs. This was previously proved in *Spodoptera exigua* NPV and NPVs from some other insects (Wang and Granados, 2000; Zhu *et al.*, 2007).

Insect PM is very similar to the cuticle of tegumentum in structure and is capable of selective permeability to the ventricular secretions and digested food particles which can easily pass through it but it inhibits the passage of indigestible compounds such as proteins and polysaccharides. In many cases, the PM role in the midgut limits the function of harmful biotic and abiotic factors of insects and entomopathogenic microorganisms such as baculoviruses. So, one of the factors limiting the effectiveness of baculoviruses, especially NPVs, is the presence of PM in the structure of the alimentary canal (Shapiro and Robertson, 1992; Wang and Granados, 2001; Zhu *et al.*, 2007). The PM thickness in insects commonly ranges from 0.1 to 2 μm (Peters, 1992), and its structure is composed

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of proteins, chitin, glycoproteins and proteoglycans (Wang and Granados, 2001). Proteins compose 35% – 55% of the PM weight (Adang and Spence, 1982) and chitin forms about 3% – 13% of it (Peters, 1992). In fact, the small quantity of chitin, compared to protein, produces a fibrous chitin network within the PM structure which acts as a physical protection against the food particles in the lumen (Wang and Granados, 2001). By affecting the chitin contents of the PM structure, optical brighteners disrupt the chitin fibril by disintegrating the bonds between proteins and chitin, which in turn, impair the PM structure and produce some gaps in it. These gaps increase the permeability of the PM to various particles including NPVs (Shapiro and Robertson, 1992; Wang and Granados, 2001). However, studies conducted on *Trichoplusia ni* larvae and some other insect species revealed that optical brighteners can effectively inhibit PM formation (Wang and Granados, 2000). So, the present investigation was undertaken to study the combination of two geographic isolates of HearNPV, collected from tomato fields of East-Azarbaijan province, Iran, with some optical brighteners to evaluate their synergistic effects on the virus against *H. armigera* larvae.

## 2 MATERIALS AND METHODS

### 2.1 Insects

A laboratory culture of *H. armigera* was maintained on a semi-synthetic diet (Shorey and Hale, 1965) based on hydrated chickpea seeds. All the experiments were carried out at the Department of Plant Protection, Faculty of Agriculture, University of Maragheh, Iran.

### 2.2 Viruses

Two HearNPV isolates used in this study were collected from tomato fields of two regions of East-Azarbaijan province, Iran, which are East-Azarbaijan-I (EAZ-I) from Maragheh region and East-Azarbaijan-II (EAZ-II) from Marand region. To prepare unique suspensions of the samples of these isolates, initial serial passages of the viral isolates ($1 \times 10^7$ OB/mL) were made in early 5th instar larvae of *H. armigera* incubated at 25 ± 1℃ (Mehrvar *et al.*, 2008b). The virus isolates were then multiplied and bioassayed in a facility away from the host culture laboratory.

### 2.3 Optical brighteners

The optical brighteners used in this study were Ranipal® produced by Pidilite Industries, Tinopal® CBS-X, Tinopal® DMA-X and Tinopal® GMS-X produced by Ciba, and Tinopal® F-3543 produced by Sigma. All the materials were powdery and diluted in distilled water and tested at 0.1% and 0.2% concentrations (w/w).

### 2.4 Bioassays

Each NPV isolate was serially diluted in distilled water to achieve the desired concentrations ranging from $5 \times 10^4$ to $0.16 \times 10^2$ OB/mL of the diet surface (with five times reduction in each treatment). Early 2nd instar larvae of *H. armigera* were released individually in the 5 mL sterilized glass vials containing semi-synthetic diet (≈1.5 mL) and incubated at 25 ± 1℃. Each treatment had 20 larvae and was replicated three times. Ten μL aliquots of each viral isolate (of different concentrations) were spread on a semi-synthetic diet using a blunt end glass rod. The virus-induced mortality was recorded daily for ten days after the inoculation. These bioassays were repeated three times for each isolate and the average was calculated. Variations in the virulence of each isolate were measured by computing the $LC_{50}$ and $LT_{50}$ values using SPSS software (version 17). Also, the relative activities and the relative speed of kill (R. S. K.) were calculated for both $LC_{50}$ and $LT_{50}$ values (Shapiro and Argauer, 2001).

In the second part of the study, the concentration of each NPV isolate was diluted in an aqueous suspension of different optical brighteners [0.1% and 0.2% (w/w)] and 10 μL of each treatment was pipetted onto the surface of 1 cm × 1 cm × 1 cm diet parts which had been previously prepared and placed in the 5 mL sterilized glass vials and allowed to air dry. The inoculated diet part was administered to an early 2nd instar larva of *H. armigera* and then incubated at 25 ± 1℃. When the diet part was completely consumed by the larva, an uninfected diet was added to the glass vial. Each treatment had 20 larvae and was replicated three times. The observations were documented for 10 days and the virus caused mortalities were identified and noted.

### 2.5 Data statistics and analysis

All data in percentage were transformed to arcsin $\sqrt{\text{percentage}}$ and then analyzed. The larval counts were also transformed to $\sqrt{x+0.5}$ values. The Probit analyses in various experiments were carried out in a Statistical Package for Social Sciences (SPSS), version 17.0 for windows. Larval mortalities in control were corrected using Abbott's correction formula (Abbott, 1925).

## 3 RESULTS

### 3.1 Biological activities of HearNPV isolates

Bioassays with HearNPV isolates against the 2nd instar larvae of *H. armigera* under laboratory conditions revealed that the EAZ-I isolate had better insecticidal activity as it achieved a lower $LC_{50}$ value of $1.98 \times 10^3$ OB/mL (Table 1). Also, the median of the lethal time of this isolate was 122.7 h (Table 3). The $LC_{50}$ and $LT_{50}$ values for the EAZ-II isolate were $2.26 \times 10^3$ OB/mL and 131.3 h, respectively (Tables 2 and 4).

### 3.2 Biological activities of HearNPV isolates in combination with optical brighteners

Bioassays to evaluate the synergistic effects of optical brighteners in combination with HearNPV isolates against the 2nd instar larvae of *H. armigera* showed that all the optical brighteners used in the study could effectively enhance virus biological activities in both levels of the application (0.1% and 0.2%). The higher levels of virus activities were achieved with 0.2% of concentrations of the chemicals. However, among the combined treatments, noticeable variations were identified in $LC_{50}$ values which ranged from $5.16 \times 10^2$ to $1.68 \times 10^3$ OB/mL, and from $6.03 \times 10^2$ to $1.99 \times 10^3$ OB/mL for EAZ-I and EAZ-II isolates, respectively (Tables 1 and 2). The lowest synergistic effect in all the cases was obtained with 0.1% Ranipal®. The main reason is probably the existence of impurities in the material which is confirmed by the company. Tinopal F-3543 in combination with EAZ-I showed the lowest $LC_{50}$ at 0.2% level ($5.16 \times 10^2$ OB/mL) meaning that Tinopal F-3543 can enhance the isolate activity 3.84 times more than that of the virus alone. Same results were also achieved by EAZ-II isolate whose activity was 3.75 times higher than that of the virus isolate alone. After the optical brighteners were added to the treatments, the median lethal time of EAZ-I and EAZ-II was significantly improved from 98.2 to 111.9 h, and from 106.3 to 119.5 h, respectively (Tables 3 and 4).

**Table 1 Probit analysis of concentration-mortality response of the 2nd instar larvae of *Helicoverpa armigera* to EAZ-I HearNPV isolate with different test materials**

| Treatments | $LC_{50}$ (OB/mL) (Fiducial limits) | Relative activity† | $\chi^{2*}$ (n−2) | Slope ± *SE* (OB/mL) |
|---|---|---|---|---|
| Virus alone (EAZ-I) | $1.98 \times 10^3$ ($1.14 \times 10^3 - 3.49 \times 10^3$) | 1.00 | 3.15 | 0.34 ± 0.05 |
| Virus + Ranipal®(0.1%) | $1.68 \times 10^3$ ($7.72 \times 10^2 - 3.22 \times 10^3$) | 1.18 | 0.91 | 0.68 ± 0.08 |
| Virus + Ranipal®(0.2%) | $1.29 \times 10^3$ ($5.53 \times 10^2 - 2.71 \times 10^3$) | 1.53 | 1.24 | 0.13 ± 0.03 |
| Virus + Tin. CBS-X (0.1%) | $1.19 \times 10^3$ ($4.35 \times 10^2 - 2.66 \times 10^3$) | 1.66 | 3.19 | 0.49 ± 0.11 |
| Virus + Tin. CBS-X (0.2%) | $7.99 \times 10^2$ ($3.26 \times 10^2 - 9.42 \times 10^2$) | 2.48 | 2.76 | 0.57 ± 0.15 |
| Virus + Tin. DMA-X (0.1%) | $1.62 \times 10^3$ ($8.37 \times 10^2 - 3.45 \times 10^3$) | 1.22 | 1.31 | 0.26 ± 0.04 |
| Virus + Tin. DMA-X (0.2%) | $1.23 \times 10^3$ ($5.69 \times 10^2 - 2.68 \times 10^3$) | 1.61 | 2.55 | 0.34 ± 0.05 |
| Virus + Tin. GMS-X (0.1%) | $1.45 \times 10^3$ ($7.93 \times 10^2 - 2.91 \times 10^3$) | 1.37 | 4.46 | 1.06 ± 0.19 |
| Virus + Tin. GMS-X (0.2%) | $9.42 \times 10^2$ ($4.17 \times 10^2 - 2.44 \times 10^3$) | 2.10 | 1.12 | 0.71 ± 0.24 |
| Virus + Tin. F-3543 (0.1%) | $7.19 \times 10^2$ ($3.12 \times 10^2 - 2.21 \times 10^3$) | 2.75 | 1.69 | 0.32 ± 0.09 |
| Virus + Tin. F-3543 (0.2%) | $5.16 \times 10^2$ ($1.68 \times 10^2 - 2.03 \times 10^3$) | 3.84 | 1.73 | 0.21 ± 0.07 |

* All lines are insignificant at $P < 0.05$. † The $LC_{50}$ values of all materials were compared with that of virus alone as the standard (Shapiro and Argauer, 2001).

**Table 2　Probit analysis of concentration-mortality response of the 2nd instar larvae of *Helicoverpa armigera* to EAZ-II HearNPV isolate with different test materials**

| Treatments | $LC_{50}$ (OB/mL) (Fiducial limits) | Relative activity† | $\chi^{2*}$ (n-2) | Slope ± *SE* (OB/mL) |
|---|---|---|---|---|
| Virus alone (EAZ-II) | $2.26\times10^{3}$ ($1.22\times10^{3}$ - $4.58\times10^{3}$) | 1.00 | 2.47 | 0.51 ± 0.04 |
| Virus + Ranipal®(0.1%) | $1.99\times10^{3}$ ($8.78\times10^{2}$ - $4.13\times10^{3}$) | 1.14 | 4.33 | 1.05 ± 0.28 |
| Virus + Ranipal®(0.2%) | $1.56\times10^{3}$ ($6.67\times10^{2}$ - $3.64\times10^{3}$) | 1.45 | 1.29 | 0.92 ± 0.21 |
| Virus + Tin. CBS-X (0.1%) | $1.44\times10^{3}$ ($6.16\times10^{2}$ - $3.89\times10^{3}$) | 1.57 | 2.56 | 0.34 ± 0.06 |
| Virus + Tin. CBS-X (0.2%) | $9.26\times10^{2}$ ($5.78\times10^{2}$ - $3.43\times10^{3}$) | 2.44 | 0.98 | 0.46 ± 0.13 |
| Virus + Tin. DMA-X (0.1%) | $1.91\times10^{3}$ ($8.25\times10^{2}$ - $4.09\times10^{3}$) | 1.18 | 3.64 | 0.75 ± 0.19 |
| Virus + Tin. DMA-X (0.2%) | $1.49\times10^{3}$ ($7.18\times10^{2}$ - $3.84\times10^{3}$) | 1.52 | 2.81 | 0.24 ± 0.07 |
| Virus + Tin. GMS-X (0.1%) | $1.74\times10^{3}$ ($7.94\times10^{2}$ - $4.03\times10^{3}$) | 1.30 | 1.69 | 0.37 ± 0.03 |
| Virus + Tin. GMS-X (0.2%) | $1.27\times10^{3}$ ($5.97\times10^{2}$ - $3.58\times10^{3}$) | 1.78 | 4.44 | 0.90 ± 0.19 |
| Virus + Tin. F-3543 (0.1%) | $8.11\times10^{2}$ ($3.67\times10^{2}$ - $3.09\times10^{3}$) | 2.79 | 1.06 | 0.26 ± 0.02 |
| Virus + Tin. F-3543 (0.2%) | $6.03\times10^{2}$ ($2.54\times10^{2}$ - $2.87\times10^{3}$) | 3.75 | 1.35 | 0.44 ± 0.06 |

* All lines are insignificant at $P>0.05$; †The $LC_{50}$ values of all materials were compared with rhat of virus alone as the standard (Shapiro and Argauer, 2001).

**Table 3　Probit analysis of time-mortality response of the 2nd instar larvae of *Helicoverpa armigera* to EAZ-I HearNPV isolate* with different test materials**

| Treatments | $LT_{50}$ (h) | Fiducial limits (h) | | Slope ± *SE* | $\chi^{2\dagger}$ (n-2) | R. S. K. (%)# |
|---|---|---|---|---|---|---|
| | | Lower | Upper | | | |
| Virus alone (EAZ-I) | 122.7 | 98.8 | 154.3 | 5.18 ± 0.20 | 4.13 | 0.00 |
| Virus + Ranipal®(0.1%) | 111.9 | 87.9 | 142.8 | 9.35 ± 0.61 | 3.97 | 8.80 |
| Virus + Ranipal®(0.2%) | 109.4 | 85.7 | 141.0 | 6.39 ± 0.23 | 6.57 | 10.84 |
| Virus + Tin. CBS-X (0.1%) | 105.2 | 81.5 | 136.2 | 8.41 ± 0.38 | 5.23 | 14.26 |
| Virus + Tin. CBS-X (0.2%) | 101.6 | 78.1 | 133.5 | 3.84 ± 0.12 | 6.48 | 17.20 |
| Virus + Tin. DMA-X (0.1%) | 110.9 | 86.9 | 142.6 | 4.71 ± 0.29 | 4.15 | 9.62 |
| Virus + Tin. DMA-X (0.2%) | 107.3 | 83.4 | 139.1 | 7.61 ± 0.37 | 5.29 | 12.55 |
| Virus + Tin. GMS-X (0.1%) | 106.5 | 83.0 | 137.4 | 5.62 ± 0.33 | 3.44 | 13.20 |
| Virus + Tin. GMS-X (0.2%) | 103.9 | 80.2 | 134.8 | 7.92 ± 0.71 | 7.29 | 15.32 |
| Virus + Tin. F-3543 (0.1%) | 100.7 | 77.3 | 131.3 | 3.23 ± 0.35 | 9.13 | 17.93 |
| Virus + Tin. F-3543 (0.2%) | 98.2 | 74.4 | 129.7 | 6.54 ± 0.49 | 4.54 | 19.97 |

* The virus concentration was $1\times10^{5}$ OB/mL; †All lines are a significantly good fit ($P<0.05$); #R. S. K. %: The $LT_{50}$ of virus alone was used as the standard and all other $LT_{50}$ values were compared with that. Percents indicate faster kill than produced by the standard (Shapiro and Argauer, 2001).

**Table 4 Probit analysis of time-mortality response of the 2nd instar larvae of *Helicoverpa armigera* to EAZ-II HearNPV isolate* with different test materials**

| Treatments | $LT_{50}$ (h) | Fiducial limits (h) | | Slope ± *SE* | $\chi^{2\dagger}$ (n-2) | R. S. K. (%)# |
|---|---|---|---|---|---|---|
| | | Lower | Upper | | | |
| Virus alone (EAZ-II) | 131.3 | 105.9 | 160.7 | 8.13 ± 0.21 | 6.67 | 0.00 |
| Virus + Ranipal®(0.1%) | 119.5 | 95.5 | 150.4 | 4.25 ± 0.23 | 6.13 | 8.99 |
| Virus + Ranipal®(0.2%) | 116.6 | 140.1 | 147.6 | 6.63 ± 0.45 | 9.44 | 11.20 |
| Virus + Tin. CBS-X (0.1%) | 113.9 | 137.9 | 143.2 | 7.74 ± 0.61 | 5.93 | 13.25 |
| Virus + Tin. CBS-X (0.2%) | 107.3 | 83.6 | 138.4 | 7.12 ± 0.41 | 3.96 | 18.28 |
| Virus + Tin. DMA-X (0.1%) | 117.8 | 92.7 | 148.5 | 5.27 ± 0.39 | 3.64 | 10.28 |
| Virus + Tin. DMA-X (0.2%) | 114.9 | 90.5 | 145.9 | 3.78 ± 0.35 | 4.52 | 12.49 |
| Virus + Tin. GMS-X (0.1%) | 113.4 | 89.4 | 144.1 | 9.21 ± 0.76 | 5.97 | 13.63 |
| Virus + Tin. GMS-X (0.2%) | 111.9 | 88.3 | 139.6 | 6.73 ± 0.54 | 3.19 | 14.78 |
| Virus + Tin. F-3543 (0.1%) | 108.1 | 83.7 | 136.1 | 8.91 ± 0.49 | 7.29 | 17.67 |
| Virus + Tin. F-3543 (0.2%) | 106.3 | 82.1 | 140.3 | 4.43 ± 0.36 | 6.98 | 19.04 |

* The virus concentration was $1 \times 10^5$ OB/mL; † All lines are a significantly good fit ($P < 0.05$); # R. S. K. %: The $LT_{50}$ of virus alone was used as the standard and all other $LT_{50}$ values were compared with that. Percents indicate faster kill than produced by the standard (Shapiro and Argauer, 2001).

# 4 DISCUSSION AND CONCLUSION

Results of this study revealed that EAZ-I isolate collected from Maragheh region of East-Azarbaijan province, showed higher viral activity compared to EAZ-II isolate. Similar differences in virulence among NPV isolates have been established in previous studies (Shapiro and Ignoffo, 1970; Hughes *et al.*, 1983; Rabindra, 1992; Arora *et al.*, 1997; Chandel *et al.*, 2004; Mehrvar *et al.*, 2007, 2008a, 2008b, 2009). However, for the effective utilization of NPV against *H. armigera*, it is crucial to develop a highly virulent isolate as a viral biopesticide.

To further enhance the biological activity of HearNPV, combinations of the highly promising adjuvant, *i. e.*, optical brighteners were also evaluated. A combination of 0.2% Tinopal F-3543 with both HearNPV isolates showed the highest virus-caused larval mortality (with the $LC_{50}$ values of $5.16 \times 10^2$ and $6.03 \times 10^2$ OB/mL for the isolates EAZ-I and EAZ-II, respectively) (Tables 1 and 2) with the lowest $LT_{50}$ values (98.2 h and 106.3 h, for EAZ-I and EAZ-II, respectively) (Tables 3 and 4) under laboratory conditions. Also, the relative speed of kill (R. S. K.), previously identified as one of the important features used to select the potent isolates (Shapiro and Argauer, 2001), was effectively enhanced by using Tinopal F-3543 for EAZ-I and EAZ-II isolates by 19.97% and 19.04%, respectively (Tables 3 and 4). R. S. K. values showed that all the optical brighteners improved $LT_{50}$ of both isolates, of which Tinopal F-3543 was identified as the most effective one. In the previous studies, optical brighteners significantly lowered the $LC_{50}$ and $LT_{50}$ values in a variety of nucleopolyhedroviruses (Shapiro and Robertson, 1992; Shapiro and Dougherty, 1994; Dougherty *et al.*, 1995; Wang and Granados, 2000; Li and Otvos, 2001; Zhu *et al.*, 2007). The present results are consistent with Zhu *et al.* (2007) studies in which M2R optical brightener improved SfaMNPV activity against *S. exigua*. They also demonstrated that M2R can impair the peritrophic membrane of the larva by increasing its permeability to the virus particles. So, this investigation can be considered as an important step in developing insect control programs by proposing PM as a target site for controlling *H. armigera* which seriously damages different crops all over the world. However, this is the first record of Iranian nucleopolyhedrovirus isolates of *H. armigera*, so future experiments can collect and make out other isolates from other parts of the country to identify the most virulent strain for further development and employment in pest management via virus enhancers, especially the optical brighteners.

To overcome the most important limitations of baculoviruses application, optical brighteners were identified as effective enhancers of the virus insecticidal activities by impairing the permeability of the PM. Results of this study showed that Tinopal F-3543 is the optimal virus enhancer as it can effectively reduce HearNPV speed of kill and its lethal concentrations. Also, the EAZ-I isolate

collected from tomato fields of Maragheh region of East-Azarbaijan, Iran, was more active compared to the other isolate, EAZ-II.

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# 荧光增白剂对伊朗核型多角体病毒分离株对棉铃虫幼虫的杀虫活性的增效作用

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**摘要**: 在室内条件下调查了几种荧光增白剂对采自伊朗 East-Azarbaijan 的棉铃虫 *Helicoverpa armigera* 核型多角体病毒两个地域株(EAZ-I 和 EAZ-II)对棉铃虫 2 龄幼虫的杀虫活性, 以提高这两个地域株的生物学活性。结果表明:与 EAZ-II 相比, EAZ-I 的杀虫活性强,其对棉铃虫幼虫的 $LC_{50}$ 和 $LT_{50}$ 值低(分别为 $1.98 \times 10^3$ OB/mL 和 122.7 h)。本研究所用的所有荧光增白剂均能有效增强病毒的生物学活性,特别是 0.2% 的 Tinopal F-3543 与 EAZ-I 混用对棉铃虫幼虫的 $LC_{50}$ 值最低($5.16 \times 10^2$ OB/mL),与病毒单独应用相比活性增强了 3.84 倍。幼虫致死的相对速率测定结果表明,荧光增白剂提高了菌株的 $LT_{50}$ 值,其中 Tinopal F-3543 的效果最佳。这些结果说明,在害虫综合治理中影响围食膜通透性的荧光增白剂与核型多角体病毒制剂混用是一种可供选择的重要方法。

**关键词**: 棉铃虫; 核型多角体病毒; 地域株; 荧光增白剂; 杀虫活性; 增效作用; 致死中时; 致死终浓度

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